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Prevalence of the Genes Encoding Propionicin T1 and Protease-Activated Antimicrobial Peptide and Their Expression in Classical Propionibacteria

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The purpose of this study was to investigate the frequency of production of the bacteriocin propionicin T1 and the protease-activated antimicrobial peptide (PAMP) and their corresponding genes in 64 isolates of classical propionibacteria. This study revealed that these genes are widespread in *Propionibacterium jensenii* and *Propionibacterium thoenii* but absent from the remaining species of classical propionibacteria that were studied. The pro-PAMP-encoding gene (*pamA*) was found in 63% of the *P. jensenii* strains and 61% of the *P. thoenii* strains, and all of these strains displayed PAMP activity. The propionicin T1-encoding gene (*pctA*) was present in 89% of the *P. thoenii* strains and 54% of the *P. jensenii* strains. All *P. thoenii* strains containing the *pctA* gene exhibited antimicrobial activity corresponding to propionicin T1 activity, whereas only 38% of the *pctA*-containing *P. jensenii* strains displayed this activity. Sequencing of the *pctA* genes revealed the existence of two allelic variants that differed in a single nucleotide in six strains of *P. jensenii*; in these strains the glycine at position 55 of propionicin T1 was replaced by an aspartate residue (A variant). No strains harboring the A variant showed any antimicrobial activity against propionicin T1-sensitive bacteria. An open reading frame (*orf2*) located immediately downstream from the *pctA* gene was absent in three strains containing the G variant of propionicin T1. Two of these strains showed low antimicrobial activity, while the third strain showed no antimicrobial activity at all. The protein encoded by *orf2* showed strong homology to ABC transporters, and it has been proposed previously that this protein is involved in the producer immunity against propionicin T1. The limited antimicrobial activity exhibited by the strains lacking *orf2* further suggests that this putative ABC transporter plays an important role in propionicin T1 activity.

Antimicrobial peptides are important components of the innate defenses in all species of organisms (8, 21). Antimicrobial peptides produced by bacteria are generally referred to as bacteriocins, which include the posttranslationally modified lantibiotics. Bacteriocins and bacteriocin-like peptides are usually ribosomally synthesized, even though it has been shown that some antimicrobial peptides from bacteria are formed by degradation of larger proteins (5, 24, 25).

The classical propionibacteria are gram-positive bacteria with a long history of safe use in dairy fermentations, especially in the production of Swiss-type cheeses, where they are responsible for the formation of flavor and the characteristic eyes. It has also been proposed that propionibacteria may function as probiotic organisms for humans and animals (2, 17).

A variety of antimicrobial compounds are produced by propionibacteria; these compounds include propionic acid, acetic acid, and diacetyl in addition to the antimicrobial peptides (9). Although a number of antimicrobial activities of propionibacteria have been reported, only three antimicrobial peptides from the classical propionibacteria have been characterized so far at the molecular level (6, 7, 14, 15, 18, 22, 26). Antimicrobial peptides from propionibacteria may have potential as natural preservatives since these organisms are considered generally recognized as safe. Propionibacteria capable of producing antimicrobial peptides may therefore be useful for safeguarding dairy products from food-borne pathogens and spoilage bacteria. Antimicrobial compounds from propionibacteria may also contribute to accelerated ripening of cheeses (6). Furthermore, bacteriocins and bacteriocin-like peptides from classical propionibacteria might function as selective weapons against the pathogenic cutaneous propionibacteria (1).

In this study we screened 64 independent isolates of classical propionibacteria in order to determine the frequencies of bacteriocin production and the corresponding genes coding for propionicin T1 (6) and the protease-activated antimicrobial peptide (PAMP) (5).

MATERIALS AND METHODS

Bacterial strains and media. The propionibacteria used in this study are shown in Table 1. The propionibacteria were propagated anaerobically in 10 ml of sodium lactate broth (SLB) (16) at 30°C for approximately 48 h. The indicator strain *Lactobacillus sakei* NCDO 2714 was propagated anaerobically in 10 ml of MRS (Oxoid) at 30°C for 24 to 48 h.

Screening for the production of propionicin T1 in agar plate assays. Strains of propionibacteria were spotted as colonies on SLB agar plates and incubated for 5 or 12 days depending on the growth rate of the strain. Five-milliliter portions SLB soft agar mixed with 0.5-ml cultures of the indicator bacterium *Propionibacterium acidipropionici* ATCC 4965 in the late logarithmic growth phase (approximately 10^8 CFU/ml) were then poured over the plates. After incubation for 48 h at 30°C, the plates were examined for zones of growth inhibition (in millimeters) surrounding the colonies.

Screening for the production of PAMP in agar plate assays. One-microliter portions of a solution containing proteinase K (20 mg/ml) were spotted at the borders of colonies of potential pro-PAMP-producing bacteria. The agar plates were then incubated for 1 to 2 h at 30°C before 5 ml of soft agar mixed with a 0.5-ml culture of the indicator bacterium *L. sakei* NCDO 2714 was poured over

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TABLE 1. Production of the antimicrobial peptides propionicin T1 and PAMP and distribution of their genes in a strain collection of classical propionibacteria

Original strain designation[a]	Original classification	Source[a]	Classification by 16S ribosomal DNA sequencing or with species-specific primers	Presence of *pctA*[b]	Antimicrobial activity against *P. acidipropionici* ATCC 4965[c]	Presence of *pamA*	Protease-activated antimicrobial activity against *L. sakei* NCDO 2714[c]
ATCC 4965	*P. acidipropionici*	ATCC	*P. acidipropionici*	−	−	−	−
ATCC 4875	*P. acidipropionici*	ATCC	*P. acidipropionici*	−	−	−	−
275	*P. jensenii*	Cow's milk, South Africa	*P. acidipropionici*	−	−	−	−
LMGT 2829	*P. acidipropionici*	Cow's milk, Europe	*P. acidipropionici*	−	−	−	−
LMGT 2831	*P. acidipropionici*	Unknown, Europe	*P. acidipropionici*	−	−	−	−
LMGT 2873	*P. acidipropionici*	Unknown, Europe	*P. acidipropionici*	−	−	−	−
ATCC 6207^{T}[d]	*P. freudenreichii*	ATCC	*P. freudenreichii*	−	−	−	−
ATCC 9614^{T} (= DSM 4902^{T})	*P. freudenreichii*	DSMZ	*P. freudenreichii*	−	−	−	−
ATCC 9616	*P. freudenreichii*	ATCC	*P. freudenreichii*	−	−	−	−
INF P203	*P. freudenreichii*	Cheese, United States	*P. freudenreichii*	−	−	−	−
INF P204	*P. jensenii*	Cheese, United States	*P. freudenreichii*	−	−	−	−
INF P205	*P. freudenreichii*	Cheese, United States	*P. freudenreichii*	−	−	−	−
INF P206	*P. freudenreichii*	Cheese, United States	*P. freudenreichii*	−	−	−	−
INF P207	*P. freudenreichii*	Cheese, United States	*P. freudenreichii*	−	−	−	−
INF P209	*P. jensenii*	Cheese, Europe	*P. freudenreichii*	−	−	−	−
LMGT 2931	*P. freudenreichii*	Dairy, Norway	*P. freudenreichii*	−	−	−	−
LMGT 2937	*Propionibacterium* sp.	Dairy, Norway	*P. freudenreichii*	−	−	−	−
LMGT 2946	*Propionibacterium* sp.	Dairy, Norway	*P. freudenreichii*	−	−	−	−
LMGT 2948	*P. freudenreichii*	Dairy, Norway	*P. freudenreichii*	−	−	−	−
LMGT 2969	*P. freudenreichii*	Dairy, Norway	*P. freudenreichii*	−	−	−	−
LMGT 3001	*P. freudenreichii*	Dairy, Europe	*P. freudenreichii*	−	−	−	−
DSM 13435	*P. microaerophilum*	Olive mill, Europe		−	−	−	−
ATCC 4868^{T}	*P. jensenii*	ATCC	*P. jensenii*	−	−	*pamA*	++
ATCC 4871	*P. jensenii*	ATCC	*P. jensenii*	−	−	*pamA*	++
ATCC 4964	*P. jensenii*	ATCC	*P. jensenii*	−	−	−	−
ATCC 14072	*P. jensenii*	ATCC	*P. jensenii*	*pctA*	+	*pamA*	++
ATCC 14073	*P. jensenii*	ATCC	*P. jensenii*	−	−	*pamA*	++
TL 207	*P. thoenii*	Dairy, Europe	*P. jensenii*	*pctA*	(+)	*pamA*	++
TL 411	*P. thoenii*	Dairy, Europe	*P. jensenii*	*pctA*	++	*pamA*	++
92	*P. thoenii*	Sludge, South Africa	*P. jensenii*	−	−	*pamA*	++
INF P311	*P. jensenii*	Cheese, United States	*P. jensenii*	−	−	*pamA*	++
INF P312	*P. jensenii*	Cheese, United States	*P. jensenii*	−	−	*pamA*	++
INF P313	*P. jensenii*	Cheese, United States	*P. jensenii*	−	−	−	−
INF P316	*P. jensenii*	Whey, Europe	*P. jensenii*	*pctA*[e]	(+)	−	−
INF P317	*P. jensenii*	Whey, Europe	*P. jensenii*	−	−	−	−
INF P318	*P. jensenii*	Cheese, Europe	*P. jensenii*	−	−	−	−
INF P319	*P. jensenii*	Sheep's milk, Europe	*P. jensenii*	*pctA*	−	−	−
INF P321	*P. jensenii*	Cheese, Europe	*P. jensenii*	*pctA*-A	−	*pamA*	++
INF P324	*P. jensenii*	Cheese, Europe	*P. jensenii*	*pctA*[e]	−	−	−
INF P325	*P. jensenii*	Cheese, Europe	*P. jensenii*	*pctA*-A	−	*pamA*	++
INF P331	*P. jensenii*	Unknown, Europe	*P. jensenii*	*pctA*-A	−	−	−
INF P332	*P. jensenii*	Unknown, Europe	*P. jensenii*	*pctA*-A	−	−	−
LMGT 2942	*Propionibacterium*	Cow's milk, Norway	*P. jensenii*	*pctA*-A	−	*pamA*	++
LMGT 2977	*Propionibacterium*	Cow's milk, Norway	*P. jensenii*	*pctA*	+	*pamA*	++
LMGT 2978	*Propionibacterium*	Cow's milk, Norway	*P. jensenii*	*pctA*-A	−	*pamA*	++
LMGT 3032	*P. freudenreichii*	Unknown, United States	*P. jensenii*	−	−	*pamA*	++
ATCC 4872	*P. thoenii*	Cheese	*P. thoenii*	*pctA*	+	*pamA*	++
ATCC 4874^{T}	*P. thoenii*	Cheese	*P. thoenii*	*pctA*	+++	*pamA*	++
TL 221	*P. thoenii*	Dairy, Europe	*P. thoenii*	*pctA*	+	*pamA*	++
288	*P. thoenii*	Sludge, South Africa	*P. thoenii*	−	−	−	−
312	*P. thoenii*	Sludge, South Africa	*P. thoenii*	−	−	−	−
419	*P. thoenii*	Cheese, South Africa	*P. thoenii*	*pctA*	+++	*pamA*	++
419-M1	*P. thoenii*	Cheese, South Africa	*P. thoenii*	*pctA*	+++	*pamA*	++
INF P409	*P. thoenii*	Cheese, United States	*P. thoenii*	*pctA*	+	*pamA*	++
INF P411	*P. thoenii*	Cheese, Europe	*P. thoenii*	*pctA*	+++	*pamA*	++
INF P412	*P. thoenii*	Unknown, Europe	*P. thoenii*	*pctA*	+++	*pamA*	++
INF P413	*P. thoenii*	Unknown, Europe	*P. thoenii*	*pctA*	+++	*pamA*	++
INF P417	*P. thoenii*	Unknown, Europe	*P. thoenii*	*pctA*	+	−	−
INF P418	*P. thoenii*	Unknown, Europe	*P. thoenii*	*pctA*	++	−	−
INF P419	*P. thoenii*	Unknown, Europe	*P. thoenii*	*pctA*	+	−	−
INF P420	*P. thoenii*	Unknown, Europe	*P. thoenii*	*pctA*	+	−	−
LMGT 2792	*P. freudenreichii*	Cheese, United States	*P. thoenii*	*pctA*	++	*pamA*	++
LMGT 2871	*P. thoenii*	Unknown, Europe	*P. thoenii*	*pctA*[e]	(+)	−	−
LMGT 2983	*P. thoenii*	Cow's milk, Norway	*P. thoenii*	*pctA*	++	*pamA*	++

[a] Abbreviations: ATCC, American Type Culture Collection (Rockville, Md.): DSM and DSMZ, Deutsche Sammlung von Mikroorganismen und Zellkulturen; INF, Department of Food Science, Agricultural University of Norway; LMGT, Laboratory of Microbial Gene Technology, Agricultural University of Norway. *P. acidipropionici* 275, *P. jensenii* 92, and *P. thoenii* 288, 312, 419, and 419-M1 are from the Environmental Bacteriology Culture Collection, University of the Orange Free State, Bloemfontein, South Africa. *P. jensenii* TL 207 and TL 411 and *P. thoenii* TL 221 are from L'Institut National de la Recherche Agronomique (INRA).

[b] *pctA* encodes propionicin T1, and *pctA*-A is a variant of the propionicin T1 gene that differs in a single nucleotide at position 257 of the *pctA* gene.

[c] (+), radius of inhibition zone, 0.2 to <1 mm; +, radius of inhibition zone, 1 to <5 mm; ++, radius of inhibition zone, 5 to <10 mm; +++, radius of inhibition zone, >10 mm. −, no PCR product or antimicrobial activity.

[d] T = type strain.

[e] *orf2* is not present.

TABLE 2. Specific primers used for PCR amplification of the 16S rRNA genes, the species-specific sequences in the 16S-23S rRNA intergenic spacer region, and the propionicin T1- and pro-PAMP-encoding genes

Primer	Sequence
16S rDNA primers	
1F	5′ GAG TTT GAT CCT GGC TCA G 3′
5R[a]	5′ GGT TAC CTT GTT ACG ACT T 3′
Primers for species-specific sequences in the 16S-23S rRNA intergenic spacer region[b]	
PacI	5′ CTG GAA GCT GGC CGT CG 3′
PacII[a]	5′ CTT GCA ACA CAA CAC ATT AC 3′
PfrI	5′ AGG AGC CTT TTC GCC ATC 3′
PfrII[a]	5′ TAG CTT GTC ACA CAA AAC TC 3′
PjeI	5′ CTA AGG AGC TGT GAC TGT G 3′
PjeII	5′ AGC TTG CAA TAC ACA CAA AAC 3′
PthI	5′ ATG GGC CCT GTG CTC AC 3′
PthII	5′ AGT AGC TTG CAA TAC ACA TAC 3′
Propionicin T1-specific primers	
PT1-PC[a]	5′ GTC TCA TGG GGT TCC CTT TTT 3′
PT1-PD	5′ CCA GGC CCG ATT CGC CCA CAG 3′
PT1-PF[a]	5′ GTA TGG CGA TGA AGA ACG AGG 3′
PT1-PG	5′ ACC TTC CAC CAA GAT CGA ACC 3′
PAMP-specific primers	
PAMP-PB[a]	5′ CAC TGA TTC CAG CGT CTG TCA 3′
PAMP-PM[a]	5′ GTA GAC CAC CGG CAG GAA GC 3′
PAMP-PP	5′ CCT TCA ACC CTA CAC TCC TCG 3′

[a] Complementary strand.
[b] See reference 28.

each plate. After incubation for 24 h at 30°C, the agar plates were examined for zones of growth inhibition (in millimeters).

DNA sequence analysis. Total DNA from propionibacteria was obtained by a freezing-heat shock method. Aliquots (1 ml) of a bacterial culture in the late logarithmic or early stationary growth phase were centrifuged for 10 min at 9,300 × *g* at room temperature. The cells were then resuspended in 100 μl of 1 mM Tris-HCl (pH 7.5)–0.1 mM Na_2EDTA (pH 8.0) and placed at −80°C for 15 min; this was followed by exposure to 100°C for 15 min. The cultures were then vortexed and frozen until they were used. Three microliters of an aliquot of lysed cells was used as the template in a PCR.

PCRs were carried out with a DNA thermal cycler (Perkin-Elmer Cetus, Norwalk, Conn.), and Taq polymerase was used as recommended by the manufacturer (Qiagen, Crawley, West Sussex, United Kingdom). The reactions (volume, 100 μl) were performed with 2.5 U of Taq polymerase and 100 pmol of each primer. The DNA primers used for amplification of the species-specific sequences in the 16S-23S rRNA intergenic spacer region (28), for the amplification and partial sequencing of the 16S rRNA genes (27), and for amplification and sequencing of the genes encoding propionicin T1 or pro-PAMP are shown in Table 2. The PCR conditions used for amplification of DNA fragments containing the propionicin T1 and pro-PAMP genes, the 16S rRNA genes, or the species-specific sequences in the 16S-23S rRNA intergenic spacer region included a hot start at 94°C for 3 min, followed by 35 cycles of denaturation at 94°C for 30 s, annealing at 58 or 60°C for 30 s, and polymerization at 72°C for 2 or 3 min.

PCR fragments were isolated by using a Qiagen PCR purification kit (Qiagen) or by agarose gel electrophoresis followed by extraction with a QIAgen gel extraction kit (Qiagen). The isolated PCR products were sequenced by using an ABI Prism dye terminator cycle sequencing ready reaction kit and an ABI PRISM 377 DNA sequencer (Perkin-Elmer, Applied Biosystems, Foster City, Calif.). Analyses of DNA and protein sequences were performed by using the OMIGA 2.0 DNA and protein sequence analysis software (Oxford Molecular, Oxford, United Kingdom).

RESULTS

Taxonomic classification of a culture collection of propionibacteria. The members of a culture collection consisting of propionibacteria obtained from various sources (Table 1) were identified to the species level by PCR amplification of the 16S-23S rRNA intergenic spacer region with species-specific primers (28) or by partial sequencing of the 16S rRNA gene and detection of species-specific sequences (27) (Table 1). The majority of the strains had up to that time been identified by conventional phenotypic classification methods. Of the 64 strains in the collection, 14 were reclassified at the species level by using the molecular methods described above (Table 1).

Distribution of the genes encoding propionicin T1 and pro-PAMP. All strains of propionibacteria in our collection were screened for the presence of the genes encoding propionicin T1 (*pctA*) and pro-PAMP (*pamA*) by performing PCRs with specific primers (Table 1). No strains of *Propionibacterium acidipropionici* or *Propionibacterium freudenreichii* harbored the genes encoding propionicin T1 or pro-PAMP. However, these genes were widely distributed among the strains of *Propionibacterium jensenii* and *Propionibacterium thoenii*. Thirteen of the 24 strains (~54%) of *P. jensenii* contained the gene encoding propionicin T1, while 15 strains (~63%) carried the pro-PAMP-encoding gene. Of the 18 strains classified as *P. thoenii*, 16 (~89%) contained *pctA* and 11 (~61%) contained *pamA*.

The structural gene of propionicin T1 in *P. thoenii* 419 and LMGT 2792 is followed by an open reading frame (*orf2*) that encodes a putative ABC transporter (6). All propionibacteria containing the *pctA* gene were tested for the presence of this gene by performing PCR with *orf2*-specific primers. With the exception of two strains of *P. jensenii* (INF P316 and INF P324) and one strain of *P. thoenii* (LMGT 2871), the expected PCR product was obtained from all strains containing *pctA*.

Expression of the propionicin T1 and PAMP activities. The antimicrobial activities of all propionibacteria in our collection were measured by the agar plate assay, and none of the strains without the *pctA* gene and without the *pamA* gene exhibited antimicrobial activity against the corresponding indicator organisms.

All strains carrying the *pamA* gene showed protease-dependent antimicrobial activity against *L. sakei* NCDO 2714 in overlay assays in the presence of proteinase K. No significant differences in antimicrobial activity were observed among the strains harboring *pamA*. In contrast, the antimicrobial activities against the propionicin T1-sensitive indicator organism *P. acidipropionici* ATCC 4965 exhibited by the *pctA*-containing propionibacteria were highly variable (Table 1). Whereas all 16 strains of *P. thoenii* harboring the *pctA* gene showed antimicrobial activity against *P. acidipropionici* ATCC 4965, only five strains of *P. jensenii* containing the gene inhibited this indicator organism (Table 1). Variation in the expression of the antimicrobial activities of propionicin T1-positive strains was also observed. The inhibition zones produced by colonies of different strains varied in size (0.5 to 12 mm) and time of production. Some strains were incubated for only 5 days before inhibition zones were noticed in the overlay assay, while other strains had to grow for 12 days before any antimicrobial activity was observed (data not shown). The remaining strains did not produce any zones of inhibition even after 20 days of growth.

Molecular analysis of the gene encoding propionicin T1. Due to the variability of the antimicrobial activities exhibited by propionibacteria containing the *pctA* gene, this gene and its

promoter region were amplified and sequenced from all these strains. The sequences revealed the existence of two allelic variants that differ in a single nucleotide (G or A) at position 257 of the *pctA* gene. This difference results in replacement of glycine by aspartate at position 55 in mature propionicin T1. The propionicin T1 gene that resulted in the aspartate substitution (A variant) was found only in strains of *P. jensenii*, and these strains did not exhibit any antimicrobial activity against the standard indicator strain (Table 1).

Previous sequencing has shown that the structural gene encoding propionicin T1 is followed by a second open reading frame (*orf2*), which encodes a putative ABC transporter. This open reading frame is most likely cotranscribed with the *pctA* gene (6). In order to detect any correlation between the presence of *orf2* and the antimicrobial activity corresponding to propionicin T1, this region was amplified by PCR with specific primers for all strains containing one of the two variants of the propionicin T1 gene.

One of the strains (*P. jensenii* INF P324) that exhibited no propionicin T1 activity although it contained the G variant of the gene gave no *orf2*-specific PCR product. Two additional strains with low levels of propionicin T1 activity, *P. jensenii* INF P316 and *P. thoenii* LMGT 2871, also lacked the *orf2* gene (Table 1).

DISCUSSION

Only a few antimicrobial peptides of the classical propionibacteria have been biochemically and genetically characterized (5, 6, 18). However, these antimicrobial compounds have several biochemical features in common, such as inhibitory spectrum, stability, hydrophobicity, and cationic nature (9).

In order to investigate the prevalence of antimicrobial peptides in propionibacteria, a collection of 64 strains was screened for the presence of the genes encoding propionicin T1 and pro-PAMP. In contrast to the gene encoding propionicin SM1 (18), these genes are probably chromosomal since the original producing strains contain no plasmids. The antimicrobial activities corresponding to these genes were also examined.

Both genes occurred at high frequencies in both *P. jensenii* and *P. thoenii*, but they were totally absent in *P. acidipropionici* and *P. freudenreichii*. Only 6 of the 42 strains belonging to *P. jensenii* and *P. thoenii* did not carry any of the genes, and only 10 strains of these species did not exhibit any antimicrobial activity. In comparison, a study in which the presence of the nisin structural gene in a collection of *Lactococcus lactis* subsp. *lactis* strains was investigated showed that about 10% of the strains carried the nisin gene (19).

All strains of *P. thoenii* containing the *pctA* gene exhibited antimicrobial activity against *P. acidipropionici* ATCC 4965, an activity that was attributed to expression of propionicin T1 (Table 1). However, only 5 of the 13 strains of *P. jensenii* containing the propionicin T1-encoding gene exhibited antimicrobial activity corresponding to this bacteriocin (Table 1). The presence of silent or nonfunctional bacteriocin genes has also been reported in sakacin P-negative strains of *L. sakei* (10) and nisin-negative strains of *L. lactis* subsp. *lactis* (19).

The variations in the production of propionicin T1 observed might simply reflect differences in growth rates between the producing strains. However, previous results also showed that propionicin T1 was produced under different growth conditions by two strains of *P. thoenii* (6). While *P. thoenii* 419 produced the bacteriocin in the late logarithmic growth phase at an incubation temperature of 30°C, propionicin T1 production by *P. thoenii* LMGT 2792 could be detected only in the stationary growth phase after incubation at 22°C (6).

To determine if the observed differences in propionicin T1 production among the *pctA*-containing propionibacteria were due to variations in the DNA sequences, the *pctA* gene from all potential producer strains was sequenced. The results of this sequencing revealed that six strains *P. jensenii* with no propionicin T1 activity harbored an allelic variant (A variant) of the *pctA* gene (Table 1). Since no differences were found in the predicted promoter regions of the allelic variants, the A variant of propionicin T1 is probably inactive or has a different antimicrobial activity spectrum.

Propionicin T1 is produced with an N-terminal leader sequence typical of peptides processed and secreted by the *sec*-dependent pathway (20, 23). The presence of a *sec* leader indicates that the limited antimicrobial activity of strains lacking *orf2* is not due to deficient peptide secretion. It has been proposed previously that the putative ABC transporter encoded by *orf2* may be implicated in propionicin T1 immunity (6). The low levels of production of propionicin T1 by the strains lacking *orf2* further support the idea that *orf2* is important for efficient production of this bacteriocin.

All of the strains of both *P. jensenii* and *P. thoenii* containing the *pamA* gene exhibited the corresponding antimicrobial activities. No significant differences in activity were observed, indicating that all strains had the same features related to the production and regulation of pro-PAMP. Previous results have shown that pro-PAMP is expressed constitutively in the producer strain *P. jensenii* LMGT 3032 (5). The activation of the exported proprotein by an external protease is an unusual feature, as most bacteriocins and antimicrobial peptides are processed and activated concomitant with export from the cell (11). However, it is possible that PAMP is the result of proteolytic degradation of a protein with a different biological function.

Phylogenetic studies have shown that *P. jensenii* and *P. thoenii* are more closely related than other dairy propionibacteria (4, 13). The strains harboring the *pctA* and *pamA* genes were obtained from different environments in Europe, the United States, and South Africa, indicating that the ability to produce these antimicrobial compounds is widely distributed in the two species. The only two strains of *P. thoenii* tested which did not contain the propionicin T1-encoding genes were isolated from sludge in South Africa (T. Langsrud, personal communication). This habitat is quite different than the habitats of most strains of propionibacteria, which are isolated mainly from various dairy sources (3, 4) (Table 1).

The inhibitory spectrum of propionicin T1 and PAMP is restricted to propionibacteria and lactobacilli (6) that occupy the same habitats as the producer bacteria. Bacteriocins are generally thought to be weapons in the competition for space and nutrients among bacteria living in the same ecological niche. Likewise, the production of propionicin T1 and PAMP might provide a selective advantage to the producing propionibacteria.

Although different species exhibit diverse inhibitory spectra in colony assays, their antimicrobial peptides can in fact be identical (12). Interestingly, we found that the producer of jenseniin G, *P. thoenii* ATCC 4872 (7), and the producer of propionicin PLG-1, *P. thoenii* ATCC 4874 (14, 15), carry both the *pctA* and *pamA* genes (Table 1). These strains also exhibited antimicrobial activities corresponding to propionicin T1 and PAMP activities, indicating that both genes were expressed. A comparison of the studies reviewed by Holo et al. (9) and the results presented here suggests that some of the previously reported antimicrobial activities of propionibacteria may be attributed to some of the peptides that have been identified already. The results described in this paper show the importance of screening potential bacteriocin producers for bacteriocin genes that already have been characterized and for the corresponding bacteriocin production in order to avoid duplication of research efforts.

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